and myosin-II (nonmuscle myosin) filaments whose formation requires the concerted action of ROCK and another Rho target, Dia1 (ref. 9). ROCK controls actomyosin filament assembly and myosin contractile activity by inducing the phosphorylation of the myosin regulatory light chain (MLC) (Fig. 1a).

How does ROCK-I activation induce blebbing? In growing cells, the expression of constitutively active ROCK mutants is known to induce the formation of thick actomyosin bundles[9], presumably by enhancing MLC phosphorylation. Coleman *et al.*[7] show that, in non-apoptotic cells, caspase-cleaved ROCK-I induces cell contraction and membrane blebbing. In addition, Sebbagh *et al.*[6] show that, after apoptosis induction, MLC phosphorylation is increased and that this requires ROCK activity. Interestingly, phosphorylation of the MLC has previously been shown to be required for membrane blebbing[3]. Taken together, these results suggest that, as a consequence of its cleavage by caspases, activated ROCK-I induces MLC phosphorylation and subsequently stimulates increased contractility (Fig. 1b). How caspase-cleaved ROCK-I alone could mediate the highly dynamic protrusion and retraction of blebs[2] is unclear, but it might be that, as plasma-membrane-associated cortical actomyosin filaments contract, they detach from the membrane focally, releasing it from restraint and allowing a bleb to protrude[2]. Perhaps other ROCK substrates, such as LIM kinase, adducin and the ezrin/radixin/moesin family[8] act in concert with MLC phosphorylation to mediate ROCK-induced blebbing.

Why does activation of ROCK-I by cleavage induce blebbing in apoptotic cells whereas Rho-activated ROCK-I induces cell contraction in non-apoptotic cells? One possibility is that, as apoptotic cells reduce their attachment to the extracellular matrix, retract and round up[1], the contractile forces generated by activated ROCK-I are not restrained by focal adhesions and the consequence is blebbing. However, cell retraction appears not to be compulsory for blebbing as expression of cleaved ROCK-I can induce blebbing in non-apoptotic cells[7] and in non-adherent Jurkat cells[6]. Another possibility is that loss of the carboxy-terminus of caspase-activated ROCK-I alters the regulation of ROCK-I, perhaps by altering its cellular localization and/or interaction with downstream effectors.

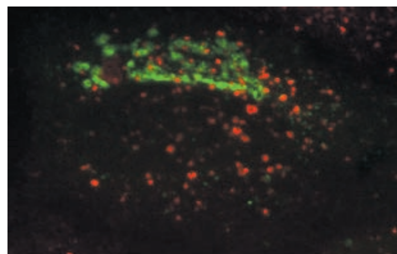
The results presented by Sebbagh *et al.*[6] and Coleman *et al.*[7] show that blebbing depends on caspase activity but not on Rho activity (Fig. 1b). In agreement with this, caspase-3-deficient cells do not bleb during apoptosis[10,11]. By contrast, others have reported that blebbing does not depend on caspase activation[3,4] but does depend on Rho activity[3]. This raises the possibility that, in certain cell types, blebbing could occur in a Rho-dependent manner (Fig. 1b), and it would therefore be interesting to determine whether ROCK-I is also involved in these situations. In addition, ROCK-I is not the only target of Rho GTPases involved in blebbing, as PAK2 has similarly been implicated in the process[12]. The p21-activated kinases (PAKs) are serine/threonine kinases that are normally activated by the Rho GTPases Rac1 and Cdc42. Like ROCK-I, PAK2 is cleaved during apoptosis, producing a constitutively active kinase fragment, and blocking PAK2 function during apoptosis interferes with blebbing. Whether cleaved PAK2 can induce blebbing on its own is not known, but it is interesting that PAK2 could phosphorylate the MLC. It will therefore be important to determine whether PAK2 cleavage contributes to increased MLC phosphorylation during apoptosis (Fig. 1b).

Stressing endocytosis

Although we have learned a great deal in recent years about the molecular machinery involved in membrane traffic, we still know relatively little about the regulation of these processes by extracellular signals. It is well established that, in many instances, ligand binding to a receptor triggers the internalization of the receptor–ligand complex and its transport through the endocytic pathway towards lysosomal degradation, recycling to the cell surface or other locations in the cell. By contrast, the bulk of endocytosis is often viewed as a constitutive process that ensures the continuous recycling of membrane components. In a recent paper (*Mol. Cell* **7**, 421–432; 2001), Cavalli and co-workers now show that a well-characterized signalling pathway that involves the MAP kinase family member p38 can regulate the rate of endocytosis by modulating the activity of the guanyl-nucleotide dissociation inhibitor RabGDI.

Rab proteins are involved in a multitude of membrane traffic and fusion events, including the endocytic pathway. They cycle between active (GTP-bound) and inactive (GDP-bound), as well as between membrane-bound and cytosolic states. The latter cycle is regulated by RabGDI, which extracts GDP-bound Rab proteins from the membrane, allowing their recycling to and subsequent activation at the next membrane. Both cycles are thought to ensure the directionality of membrane traffic.

Cavalli *et al.* searched for an upstream regulator of RabGDI that would increase its capacity to extract Rab5—a Rab protein involved in early steps of endocytic transport—from endosomal membranes. The activity they identified was stress-activated p38 kinase, which directly phosphorylates serine 121 of RabGDI. In intact cells, the p38 pathway can be activated by various cellular stresses, such as oxidative stress or UV irratiation. Under stress situations, Rab5 and its effector EEA1 were released from endosomes, and this depends on the presence of serine 121 in RabGDI. The image shows that the association of EEA1 (red) with endosomes is resistant to H_2O_2-induced release in the presence of the RabGDI S121A mutant (co-transfected with a GFP construct (green) to label transfected cells). Most importantly, exposure of cells to UV light or H_2O_2 increased the rate of endocytosis in normal cells but not in cells lacking p38. Furthermore, basal levels of p38 activity seem to be required for the basal rate of endocytosis. Taken together, these data point to the existence of a pathway that links p38 and RabGDI to both constitutive endocytic membrane transport and its regulation in response to external stress stimuli.



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But why increase the rate of endocytosis in times of stress? Cavalli *et al.* speculate that the increase in endocytosis in response to extracellular stresses might allow more efficient internalization of cell surface components for repair, storage or degradation. Although this intriguing hypothesis requires further support, this study provides a provocative start in identifiying the molecular mechanisms through which fundamental membrane trafficking pathways can be regulated by environmental stimuli. It is likely that more links between extracellular signals and the basic membrane traffic machinery will be unveiled in the future.

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